CE

Product Description Specific IgE

Product and Process Description

Determination of specific immunoglobulin E (IgE) in serum or plasma is based on the principle of an enzyme immunoassay (EIA) for quantitative analysis of allergen specific IgE in serum or plasma. Specific IgE determination performed with this testing kit is only validated in combination with BDL testing system und must not be performed with other systems, because investigated performance data have been validated especially for BDL testing systems. Testing application is restricted to qualified personnel that are specially trained in applying IVD operations.

IgE is a serum protein and main carrier of reagin activity of allergic type I reactions (immediate-type allergy). IgE circulates in blood stream; being responsible for clinical symptoms of type I reactions; IgE binds to the surface of mast cells and basophilic granulocytes. Attachment occurs through the Fc part of the IgE molecule. Allergen contact with corresponding (specific) IgEs leads then to release of pro-inflammatory mediators and hormones (e.g. histamine). Using the test procedure for circulating specific IgE, cell-attached IgE cannot be determined. Therefore results coming from specific IgE determination in serum should be only a part of a diagnose concept which consists of an accurate anamnesis, skin prick test and provocation test as well as other *in-vitro* assays.

Quantitative determination of circulating specific IgE results from a non-competitive enzyme immunoassay. The solid phase consists of a chemically activated paper disc, which contains the corresponding, covalently bound

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allergens.

First operation step contains pipetting of patient serum or plasma onto the paper disc. Here a bond between allergen specific IgE and the allergen as solid phase develops. Excessive serum is removed by washing. As second step an enzyme-linked anti-human IgE is pipetted onto the paper disc. Unbound conjugated anti-IgE is removed via a washing step. The amount of bound enzyme-linked anti-human IgE is proportional to the amount of specific IgE in serum / plasma. Next operation step includes addition of substrate solution (p-nitrophenyl phosphate, PNPP). Caused by the activity of the linked enzyme (alkaline phosphatase) substrate is processed and a coloured solution develops. Enzyme reaction is abandoned via addition of stopping solution at the end of incubation time. Extinction of the coloured solution is investigated by photometric measurement. Data analysis is performed by using a standard curve consisting of extinction values of measured standard cavities. Figure 1 illustrates all operation steps and underlying molecular background.

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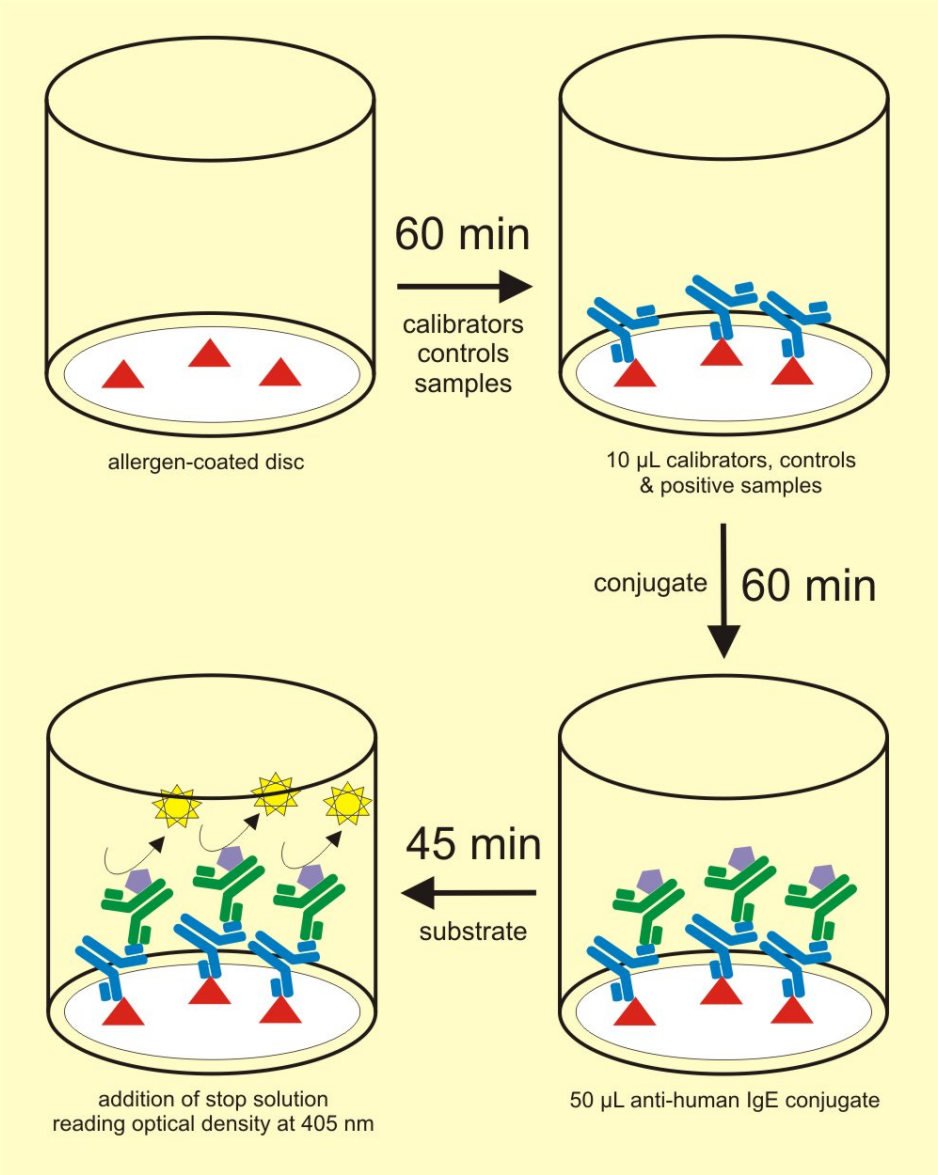

Fig.1: Specific IgE assay procedure

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Assay Limitations

Reliable and reproducible results can only be received in terms of a testing procedure that corresponds to the specifications of the product.

In case of using several microwell cavities in one test, incubation times of individual plates must be considered.

Especially in terms of food allergies negative *in-vitro* results may occur, although distinct clinical symptoms are present. This phenomena result from maturation, industrial processing, cooking, roasting, etc., but also from digestion. Thus *in-vivo* there may be totally different protein structures compared to proteins bound to the solid phase of the assay. Furthermore a set of food products are quite sensitive leading to less binding efficiency of native-structured proteins to the solid phase.

For *In-vitro* determination of haptens human serum albumin (HAS) is used as spacer substance. This realises reproducible manufacturing of a virtual full-antigen for *in-vitro* analysis. This procedure of course cannot fully image complete reaction possibilities in the human body. Thus *in-vitro* assay may not lead to positive results in every case of patients that reveal positive clinical results.

In general negative results for insect venoms only indicate that currently no circulating specific IgE against tested venoms is detectable in serum or plasma. Therefore it cannot be concluded that the patient will not develop clinical symptoms in case of a present or prospective bite. Considering insect venoms a temporary consumption of antibodies may occur after exposition has happened for a couple of time.

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Negative in-vitro results can occur if:

- symptoms are not IgE mediated
- sample was taken before organism could generate antibodies against allergen
- IgE level could regenerate after long period of sensitisation
- IgE consumption due to allergic reaction (venoms)

Identical results of different patients do not come along with the same reaction basis because of individual difference.

Positive results in specific IgE *in-vitro* tests may not mandatorily be accompanied by clinical manifestations.

Many IgE antibodies exhibit cross reactivities to other IgE antibodies, e.g. birch pollens, apple, mugwort, celeriac, latex or banana. Diagnosis must consider circumstances of the specific case.

Production procedure

Manufacturing of all components belonging to the testing system follow validated standard operation protocols and underlie strict quality guidelines implemented by BDL Labordiagnostik GmbH in accordance with current authority requirements. Every component passes appropriate quality controls before approval.

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